—Editor's note: As John mentioned in his article, he has been distributing this cultivar for many years. Growers with gigantic *Cephalotus* specimens can be quite confident it is the same clone that John described in his article. John is busy with his fieldwork and collection, so is unable to send people specimens of this cultivar, but if you want it—look around. It is increasingly common in collections. (BAMR)

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Technical Refereed Contribution

Medium Optimization for Growing *Aldrovanda vesiculosa* in vitro

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Kondo *et al.* (1997) reported that Japanese and Polish strains of *Aldrovanda vesiculosa* can be grown in a sterile in vitro culture. They used Gamborg B5 liquid medium (Gamborg *et al.*, 1968) with 2% sucrose. While the concentrations of nutrients such as NH_4^+, NO_3^-, PO_4 (and sometimes also K^+) are very low in both *Aldrovanda* habitats (Kaminski, 1987; Adamec, 1999a) and in outdoor cultivation tanks (Adamec, 1997), the Gamborg B5 liquid medium Kondo *et al.* (1997) used is very concentrated (in mg l^{-1}: KNO_3, 2500; $(NH_4)_2SO_4$, 134.0; NaH_2PO_4, 130.5; $CaCl_2.6H_2O$, 223.5; $MgSO_4.7H_2O$, 250.0; $FeSO_4.7H_2O$, 27.8; Na_2EDTA, 37.3; H_3BO_3, 3.0; $MnSO_4$, 10.0; $ZnSO_4.7H_2O$, 2.0; $CuSO_4.5H_2O$, 0.025; $CoCl_2.6H_2O$, 0.025; $Na_2MoO_4.2H_2O$, 0.25; KJ, 0.75; inositol, 100; thiamine, 10.0; nicotinic acid, 1.0; pyridoxine, 1.0). This solution is not ideal for *Aldrovanda*: the very high KNO_3 concentration is unnecessary since *Aldrovanda* only slightly takes up NO_3^- ions (Adamec, 2000), and the boron concentration might be dangerously close to toxic (cf. Adamec, 1999b). We tried to find an improved substitute for the B5 medium.

The following modifications of the B5 were tested first: A)100% B5 (as a control); B)75% B5; C)50% B5; D)100% B5 but KNO_3 only 500 mg l^{-1}; E)75% B5 but KNO_3 500 mg l^{-1}; F)50% B5 but KNO_3 500 mg l^{-1}. All modifications contained 2% sucrose, and their initial pH was set at approximately 5.5 before autoclaving at 125°C for 20 min. To investigate a possible role of tannins for *Aldrovanda* growth in vitro (Kaminski, 1987) each trial also had a variant that was supplemented with sedge litter as a natural source of tannins; (approximately 200 mg dry weight of dead brown *Carex gracilis* leaves per 100 ml of medium in 150-ml Erlenmeyer flasks were added before autoclaving). These media were slightly brownish, indicated the presence of tannins and/or humic acids. One plant of the Japanese strain in vitro with 1-2 apices was aseptically transferred to each trial medium. The media were not shaken. After 34 days of growth at 25-28°C under fluorescent lighting (14 h light/10 h darkness), plant growth was evaluated on the basis of plant size, structure, and color. No effect of sedge litter was observed, so the requirement of humic acids or tannins for *Aldrovanda* growth in the presence of sucrose and inositol is zero. Of all the trials, the plants in group A (the control) were the worst: the plants were smallest with small traps, some leaves were damaged and blackened. The plants at D and E were bigger and healthy, and those in B and C were even larger. The best (biggest

plants with big traps) were in F. These plants were 6-8 cm long.

Thus, of all the media tested, the best (F) was half-strength B5 with only 500 mg l^{-1} KNO_3. In contrast, the full-strength medium was slightly toxic. The plants in all the trials branched poorly. It was obvious that they only branched during a limited period, just after the transfer to new media. The pH of the old B5 medium from which the plants were transferred was only 3.2 and 3.4. Similarly, after the experiment, we measured pH of 3.1 in D and 3.4 in F medium. Since the uptake of NH_4^+ by plants leads to medium acidification, while that of NO_3^- to alkalization the strong acidification of the media measured confirms that NO_3^- uptake is unimportant. It is probable that very low pH in older media prevents the plants from branching.

In the second run, we tested the effect of dilution of the B5, KNO_3, and microelements (MI). Three apices were transferred to each Erlenmeyer flask. The growth conditions were the same as above. Since the growth period lasted 79 days the final cultures were too dense. The following modifications of the B5 were tested, and the results after the final growth phase are given below:
50% B5+500 mg l^{-1} KNO_3+50% MI: the best variant, mean 20.0 apices per flask, big and healthy apices, final pH 3.15;
25% B5+500 mg l^{-1} KNO_3+50% MI: bad variant, mean 10.3 apices, apices usually small, final pH unmeasured;
10% B5+500 mg l^{-1} KNO_3+50% MI: the second worst variant, mean 6.3 apices, apices usually small, final pH 3.41;
50% B5+250 mg l^{-1} KNO_3+50% MI: mediocre variant, mean 11.0 apices, apices bigger, final pH unmeasured;
50% B5+100 mg l^{-1} KNO_3+50% MI: the worst variant, mean 13.0 apices, small yellowing plants, final pH 2.94;
50% B5+500 mg l^{-1} KNO_3+25% MI: the second best variant, mean 18.3 apices, apices big, final pH unmeasured.

In the second tests, the best modification of B5 was the same as in the first set of tests: half-strength B5 with 500 mg l^{-1} KNO_3. We denote it as "standard medium". Thus, any further dilution of both macro- and microelements led to impairing *Aldrovanda* growth. It follows that KNO_3 concentration greatly influences the final pH of the media. At low [KNO_3], pH can fall well below 3.0 which, obviously, is far from the optimum. The "standard medium" has also shown to be quite suitable for red Australian *Aldrovanda* strains.

We have also found that the "standard medium" is also very suitable for growing terrestrial *Utricularia* (*U. longifolia, U. monanthos, U. humboldtii,* etc.) and *Genlisea* species (e.g., *G. aurea, G. violacea*) in vitro. In comparison with the results from the media often recommended for carnivorous plants (e.g., 30-50% the concentrations recommended by Murashige & Skoog (1962) solidified with agar), the growth of the above species in the "standard medium" is very rapid and vigorous.

We observed that when *Aldrovanda* plants from in vitro culture were transferred back to a nutrient-poor water in an aquarium or container their growth was very rapid for the following weeks. We suspected that the plants growing in vitro had very high nutrient content in their tissues. However, the elemental content in % of dry weight found in apices of young, in vitro grown Japanese plants was about the same as that in Polish plants growing vigorously in an outdoor culture (the values in parentheses are from Adamec (2000) for outdoor culture; see this paper for further experimental details): N, 1.24 (1.31); P, 0.48 (0.48); K, 2.21 (1.86); Ca, 0.24 (0.17); Mg, 0.11 (0.16). Since the mineral content of the in vitro plants are not elevated, it is possible that instead, the accelerated growth is due to an exaggerated richness sugar resources. These sugar resources might enhance the growth for a few weeks after a transfer to nutrient-poor and sucrose-free water.

Some carnivorous plant growers have tried to prepare a sterile in vitro culture of *Aldrovanda* by seed sterilization. When we attempted this, we encountered two prob-

lems. After a conventional seed sterilization (4.8% sodium hypochlorite for 25 minutes using 153 seeds from Polish and Romanian plants) we found a positive correlation between seed germination and contamination in various seed test groups (in 30% B5 + 75 mg/l KNO_3 + 2% sucrose; 14 h L/10 h D photoperiod). In all, 44.4% of the seeds showed signs of germination and 52.3% of the seeds were contaminated. Seed contamination mostly occurred at the micropylar end of the seeds after they had started germinating. So, most of ripe *Aldrovanda* seeds maintain microbial infection close to the lid (operculum), i.e., in the micropylar hole, even after a strong sterilization. In sterilized seeds, the infection is released mostly after the first signs of seed germination when the emerging radicle tears off the black lid (this stage is visible by naked eye; e.g. Kondo *et al.*, 1997; Daly, 1998). However, the emergence of the radicle usually ceased at this initial stage and germination stopped. Although Kondo *et al.* (1997) succeeded in introducing European *Aldrovanda* into in vitro cultivation, we conclude that this method is not sufficiently reliable. Instead, we recommend starting with young defoliated shoot segments and applying a mild surface sterilization.

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